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1. Microbiological studies were completed on the Apollo 17 spacecraft: a total of 435 microorganisms were isolated and identified from the Command Module (CM), and approximately 50% of the isolates were obtained from the interior surfaces at pre-flight. Table 1 shows a comparison of types of microorganisms detected in pre- and post-flight samples.

   The types of microorganisms detected on pre- and post-flight samples using various media and incubation conditions are listed in Tables 2 and 3. A total of 20 types or groups of microorganisms were identified on pre-flight (Table 2) and 14 on post-flight samples (Table 3). Staphylococcus aureus (Subgroup I) was isolated on both pre- and post-flight samples. On pre-flight, an organism belonging to the Streptococcus-Enterococcus group was isolated; this group is not typically found on spacecraft surfaces. Gram-negative microorganisms were not recovered on either pre- or post-flight samples even when Eosin-Methylene-Blue Agar was used to ameliorate recovery. Molds (Penicillium, sp.) were recovered on pre- and post-flight samples and yeasts were isolated only on pre-flight.

   The majority (97%) of microorganisms detected on pre-flight samples were types considered indigenous to human hair, skin, and respiratory tract. The occurrence of microorganisms associated with soil and dust in the environment were low. These results are comparable to those obtained from previous CM samplings.

   All data pertaining to enumeration and identification of microorganisms from the Apollo spacecraft were treated and stored on a CDC 3600 computer at Cape Kennedy for rapid retrieval. Computer printouts were compiled and sent to the Planetary Quarantine Officer.

2. Prior to launch, a microbiological profile was performed on the outbound automated Pioneer G spacecraft at Cape Kennedy. Twenty-six sites, each consisting of 4 square inches were assayed, and quantitative results were submitted to the Planetary Quarantine Officer. Bacterial colonies were picked from TSA plates and are in the process of being identified; qualitative results will be reported next quarter.

3. The study to compare the numbers and types of molds from TSA pour plates with those isolated on other media was concluded. Cornmeal + Chloromycetin spread plates have been shown to give the best quantitative results for isolation of molds from spacecraft surfaces (Q.R. #40). A total of 422 identifications were made. Dr. Brandsberg of the Kansas City Laboratories identified 299 isolates and an additional 123 were identified at the Planetary Quarantine Laboratory at Cape Kennedy. Table 4 shows the genera and numbers observed from each type of media. For qualitative comparison of the isolation media, results from the Instrument Unit and Saturn S4B modules were consolidated. A total of 51 different genera were observed from all media; twelve genera were observed on TSA pour plates, twenty-three on TSA spread plates, twenty-seven on Mycophil + Chloromycetin and thirty-four on Cornmeal + Chloromycetin. These represent 24%, 45%, 53%, and 67% of the genera observed, respectively. The Mycophil + Chloromycetin and Cycloheximide plates used to detect slow

growing molds yielded only one isolate not observed on other media. This was Scopulariopsis, a mold not considered to be a slow grower. Cornmeal Agar + Chloromycetin appears to be the medium of choice for both quantitative and qualitative isolation of molds from spacecraft surfaces.

4. Studies to examine the possibility of shifts in biochemical reaction patterns during storage and subculture of environmental Bacillus isolates (Q.R. #37, 39) were continued. Thirty environmental Bacillus, sp. isolates from the MSOB clean room and eleven standard cultures obtained from the ATCC or NRS (Dr. R. Gordon's collection) were subjected to different types of subculture and storage prior to identification. Cultures were initially identified after two subcultures on TSA using the version III Bacillus identification scheme (Q.R. #39). The culturing and storage methods were as follows:

    1. Two sequential (24 hr) subcultures on TSA and storage at 4 C for 5 weeks.
    2. Ten sequential subcultures on TSA (twice weekly for 5 weeks; cultures identified after 5 and 10 subcultures; cultures were at 32 C at all times).
    3. Cultures from 1. above were subcultured after 5 weeks and treated as 2. above.
    4. Cultures from 2. above were subcultured and treated as 1. above.

Changes in biochemical characters due to subculture and storage are shown in Table 5. Variations were observed in all biochemical tests regardless of the type of manipulation. To determine the number of deviations, the results from three replicate biochemical tests were treated as one, and then compared to previous test results.

The 41 cultures which had been stored and identified on two separate occasions showed 82 test deviations. Cultures which had been subcultured twice weekly, and identified on 4 separate occasions showed 165 test deviations. An analysis of the data revealed an average of one deviation per culture per identification (I.D.). The range of deviations per culture was 0 to 5 per I.D. with most I.D. having at least one deviation. These results show that an unknown culture of Bacillus may be expected to yield test reactions that deviate slightly from an I.D. scheme and that the tests which may change cannot be predicted for any specific organism. Consequently, multiple transfers and cold temperature storage, as described, does not appear to significantly affect identifications. From a practical viewpoint, this means that unknown Bacillus cultures differing from the I.D. scheme by 1 or 2 deviations can be assigned definite species names with assurance and not be classified as Atypical Bacillus.

Spore morphology has been used for the separation of Bacillus into three groups. This criterion was tested to determine if spore morphology would be a practical method for the identification of Bacillus, spp. Twenty-six ATCC or NRS Bacillus type cultures representing 14 species were chosen to observe spore morphology. The cultures were grown on TSA slants

TABLE 1. COMPARISON OF THE TYPES OF MICROORGANISMS DETECTED FROM THE APOLLO 17 COMMAND MODULE

	Pre-Flight (T-9 Hours)	Post-Flight
Staphylococcus spp.		
Subgroup I	+	+
Subgroup II	+	+
Subgroup III	+	+
Subgroup IV	+	+
Subgroup V	+	+
Subgroup VI	+	+
Micrococcus spp.		
Subgroup 1	+	+
Subgroup 3	+	-
Subgroup 5	+	-
Subgroup 6	+	-
Subgroup 7	+	+
Streptococcus-Enterococcus Group	+	-
Streptococcus-Viridans Group	-	+
Bacillus spp.		
B. cereus	+	-
B. coagulans	+	-
B. pumilus	+	-
B. subtilis	-	+
Corynebacterium-Brevibacterium Group	+	+
Yeasts	+	-
Molds	+	+
Atypical *Micrococcus* spp.	+	+
Atypical *Bacillus* spp.	+	+
Number Isolated	222	213

TABLE 2. TYPES OF MICROORGANISMS DETECTED ON PRE-FLIGHT (T-9 HOURS) FROM THE COMMAND MODULE OF APOLLO 17 ON VARIOUS MEDIA

	INCUBATION CONDITIONS					
	Aerobic				Anaerobic	
	TSA	BA	BAS	EMB	BA	BAS
Staphylococcus spp.						
Subgroup I	+	+	+	-	+	-
Subgroup II	+	+	+	-	+	+
Subgroup III	+	+	+	-	-	-
Subgroup IV	+	+	+	-	+	+
Subgroup V	+	-	+	-	+	+
Subgroup VI	+	+	+	-	+	+
Micrococcus spp.						
Subgroup 1	+	-	+	-	-	-
Subgroup 3	+	-	-	-	-	-
Subgroup 5	+	-	-	+	-	-
Subgroup 6	+	+	-	-	-	-
Subgroup 7	+	-	+	+	-	-
Streptococcus-Enterococcus Group	+	-	-	-	-	-
Bacillus spp.						
B. cereus	+	-	-	-	-	-
B. coagulans	-	+	-	-	-	-
B. pumilus	-	-	+	-	-	-
Corynebacterium-Brevibacterium Group	+	+	+	+	+	+
Yeasts	-	+	-	-	-	-
Molds	+	-	-	-	-	-
Atypical *Micrococcus* spp.	+	+	+	+	-	-
Atypical *Bacillus* spp.	+	+	+	-	-	-
Number Isolated	96	47	41	7	15	16

TSA - Trypticase Soy Agar
BA - Blood Agar
BAS - Blood Agar enriched with vitamin K and Hemin
EMB - Eosin-Methylene Blue agar

and observed periodically for sporulation. Slides were prepared by making smears from the slant, allowing to air dry and staining for one minute with crystal violet. The smears were not heat fixed. Spore morphology was observed with a Zeiss Universal Microscope under oil immersion and phase contrast using a green contrast filter. Slides that showed mature spores encased in their sporangia were photographed in black and white with a Polaroid camera attached to the microscope. A total of 40 photographs were taken. These photographs were coded and placed into three groups according to Dr. Gordon's new monograph on the Genus Bacillus. The groups are as follows:

Group I: Sporangia not definitely swollen; spores ellipsoidal or cylindrical, central to terminal; Gram-positive.
Group II: Sporangia swollen by ellipsoidal spores; spores central to terminal; Gram-positive, -negative, or -variable.
Group III: Sporangia swollen; spores generally spherical, terminal to subterminal; Gram-positive, -negative, or -variable.

The photographs were uncoded and checked to see if they were correctly placed as shown.

Placement of Photographs

	No. Correct	No. Incorrect
Group I	12	1 (Belongs in Group II)
Group II	12	11 (Belong in Group I)
Group III	4	0

In Group III, only one representative species was studied (B. sphaericus) and 4 photographs of two cultures from this species were correctly placed. In Group II, 12 photographs of 10 cultures from 6 species were correctly identified. One photograph belonging to a member of Group II was incorrectly identified. In Group I, 12 photographs of 8 cultures from 4 species were correctly identified. Eleven photographs of 7 cultures from 7 species belonging to Group I were incorrectly placed into Group II. In some cases, more than one photograph was taken of the same organism. These multiple photographs were matched correctly in all except one case.

These results seem to indicate that identification by spore morphology, even into only three groups, is not practical. Group I with sporangia not definitely swollen and Group II with sporangia swollen is not a distinct and easily detected difference. Approximately 50% of the photographs of Group I isolates showed sufficient sporangial swelling that they were incorrectly placed into Group II.

5. A study was conducted to determine the relationship between the types and levels of Bacillus, spp. found in a clean room with that outside the controlled environment. Air samples were taken in three different but adjacent

environments: (1) Clean room in the Manned Space Operations Building (MSOB), (2) Corridor outside the clean room, and (3) Outdoors. Membrane filter (MF) field monitors and stainless steel fallout strips (SSS) were employed. Isolates were enumerated and identified. Table 6 shows the levels and types of Bacillus, spp. identified from each area.

The data concerning the types and levels of Bacillus, spp. in the clean room, corridor and outside at Cape Kennedy were studied and analyzed. Attempts were made to find a correlation between levels in the clean room, corridor and outside by calculating correlation coefficients for the various pairs. All coefficients were near zero indicating a total absence of correlation in any of the comparisons.

The data relating to types of Bacillus, spp. were also analyzed by calculating correlation coefficients for the frequency of occurrence of each species listed. This test essentially answered the question, "To what degree are the most common species in one area also the most common species in another area and the least common in one area also the least common in another area?" The following comparisons were made and the resulting r values calculated.

Comparison	r value
Clean room SSS vs. Clean room Air	0.890
Clean room SSS vs. Corridor Air	0.972
Clean room SSS vs. Outside Air	0.980
Clean room Air vs. Corridor Air	0.849
Clean room Air vs. Outside Air	0.868
Corridor Air vs. Outside Air	0.961

The r values express the degree of correlation with 1.000 being perfect correlation and 0 being no correlation. Each comparison involved at least 12 species for which there were data. The r value at the 99% confidence level for this number of observations is .68 so it is apparent that the correlation observed for each comparison is statistically significant. In fact, the highest degree of correlation was between clean room SSS and outside air. While these correlations have been suspected for years by environmental microbiologists this may constitute the first experimental demonstration of the phenomena.

6. The terminal sterilization process for unmanned lander spacecraft is continuing. Thermal inactivation experiments are presently being conducted using the proposed thermal profile. Results of these experiments will be reported in the next quarter.

TABLE 3. TYPES OF MICROORGANISMS DETECTED ON POST-FLIGHT FROM THE COMMAND MODULE OF APOLLO 17 ON VARIOUS MEDIA

	INCUBATION CONDITIONS					
	Aerobic				Anaerobic	
	TSA	BA	BAS	EMB	BA	BAS
Staphylococcus spp.						
Subgroup I	+	-	-	-	+	-
Subgroup II	+	+	+	-	-	-
Subgroup III	+	-	-	-	-	-
Subgroup IV	+	-	-	-	-	-
Subgroup V	+	+	+	-	-	-
Subgroup VI	+	+	+	-	+	-
Micrococcus spp.						
Subgroup 1	+	-	+	-	-	-
Subgroup 7	+	+	-	-	-	-
Streptococcus-Viridans Group	-	+	-	-	-	-
Bacillus spp.						
B. subtilis	-	-	+	-	-	-
Corynebacterium-Brevibacterium Group	+	+	+	+	+	+
Molds	+	-	-	-	-	-
Atypical *Micrococcus* spp.	+	-	-	-	+	-
Atypical *Bacillus* spp.	+	-	-	-	-	-
Number Isolated	180	14	12	1	5	1

TSA - Trypticase Soy Agar
BA - Blood Agar
BAS - Blood Agar enriched with vitamin K and Hemin
EMB - Eosin-Methylene Blue agar

TABLE 4. GENERA AND TYPES OF MOLDS ISOLATED ON VARIOUS MEDIA

Genera or Type	TSA Pour	TSA Spread	Myco C[1]	Corn C[2]	Myco C&A[3]	Genera or Type	TSA Pour	TSA Spread	Myco C[1]	Corn C[2]	Myco C&A[3]
Curvularia	+	+	+	+	-	Piggotia	-	-	+	+	-
Penicillium	+	+	+	+	-	Torula	-	-	+	-	-
Aspergillus	+	+	+	+	-	Cytosporella	-	-	+	-	-
Spegazzinia	+	+	+	+	-	Cytospora	-	-	+	-	-
Trichoderma	+	+	-	-	-	Helminthosporium	-	-	+	-	-
Chaetomium	+	+	+	+	-	Leptosphaeria	-	-	+	-	-
Thielavia	+	+	-	-	-	Cephalosporium	-	-	+	+	+
Fusarium	+	+	-	+	-	Chaetomella	-	-	+	-	-
Bipolaris	+	+	+	+	-	Aureobasidium	-	-	+	+	-
Streptomyces	+	-	-	-	-	Calcarisporium	-	-	-	+	-
Monocillium	+	-	-	-	-	Humicola	-	-	-	+	-
Rhizopus	+	+	+	+	-	Oidiodendron	-	-	-	+	-
Cladosporium	-	+	+	+	+	Alternaria	-	-	-	+	-
Asperisporium	-	+	-	-	-	Monodyctis	-	-	-	+	-
Periconiella	-	+	+	+	-	Chitonospora	-	-	-	+	-
Paecilomyces	-	+	+	+	-	Pseudobotrytis	-	-	-	+	-
Nigrospora	-	+	+	+	-	Mammaria	-	-	-	+	-
Sphaeropsis	-	+	-	-	-	Coniochaeta	-	-	-	+	-
Pucciniopsis	-	+	+	-	-	Papularia	-	-	-	+	-
Mucor	-	+	+	+	+	Epicoccum	-	-	-	+	-
Chaetophoma	-	+	-	-	-	Phialophora	-	-	-	+	-
Pestalotia	-	+	+	+	-	Scopulariopsis	-	-	-	-	+
Pithomyces	-	+	+	+	-	Phycomycete	-	-	-	+	-
Rhinocladium	-	+	-	-	-	Basidiomycete	-	-	-	+	-
Phoma	-	-	+	+	-	Sterile mycelium	-	+	+	+	+
Geotrichum	-	-	+	-	-						
					TOTAL 51		12	23	27	34	6
					Percentage of Total		24%	45%	53%	67%	12%

[1]Mycophil with Chloromycetin
[2]Cornmeal with Chloromycetin
[3]Mycophil with Chloromycetin and Acti-dione

TABLE 5. CHANGES IN BIOCHEMICAL TESTS OF BACILLUS MICROORGANISMS AFTER TWO TYPES OF CULTURE HANDLING PRIOR TO IDENTIFICATION

Treatment	Number of Test Cultures	Deviations from Expected Reactions[a]									
		Starch	Casein	P.R. Mannitol	VP	Citrate	Nitrate	Anaerobic Growth	Tyrosine	Phenyl-alanine	Total Deviations
Stored 5 weeks (I.D. 2 times)	41	6	8	6	13	5	4	22	9	9	82
Transferred 10X (I.D. 4 times)	41	11	9	13	23	18	13	42	21	15	165

[a]Deviations are based on the results from 3 replicate biochemical tests.

TABLE 6. *BACILLUS* SPP. ISOLATED AND IDENTIFIED FROM THREE DIFFERENT BUT ADJACENT ENVIRONMENTS

	Cleanroom		Corridor		Outdoors		SSS in Cleanroom	
	#	%	#	%	#	%	#	%
B. alvei	5	2.91	5	1.99	10	5.07	4	1.90
B. cereus	15	8.72	34	13.54	21	10.66	19	9.01
B. circulans	9	5.23	2	0.80	1	0.51	4	1.90
B. coagulans	1	0.58	ND	ND	1	0.51	2	0.95
B. firmus	6	3.49	6	2.39	2	1.02	5	2.37
B. laterosporus	ND	ND	1	0.40	ND	ND	1	0.47
B. licheniformis	6	3.49	15	5.97	10	5.07	13	6.16
B. macerans	1	0.58	1	0.40	1	0.51	4	1.89
B. megaterium	25	14.53	57	22.71	45	22.84	45	21.33
B. polymyxa	ND	ND	1	0.40	ND	ND	ND	ND
B. pumilus	23	13.37	19	7.57	17	8.63	17	8.06
B. subtilis	16	9.30	26	10.36	17	8.63	23	10.90
B. sphaericus	2	1.16	1	0.40	ND	ND	ND	ND
B. lentus	9	5.23	13	5.18	13	6.60	13	6.16
B. brevis	19	11.05	20	7.97	11	5.58	14	6.63
Atyp. *Bacillus*	35	20.35	50	19.92	48	24.37	47	22.27
TOTAL	172		251		197		211	

ND - None detected